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TEMPORAL RESOLUTION OF GENERAL ODOR PULSES BY OLFACTORY SENSORY NEURONS IN AMERICAN COCKROACHES

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Summary

Behavioral and physiological evidence indicates that insect pheromone sensory neurons are able to resolve pulses of pheromone concentration as they occur downwind from a point source, but the abilities of insect sensory neurons that are sensitive to general odors to respond to pulsatile stimuli are unknown. The temporal response characteristics of olfactory sensory neurons of female American cockroaches *Periplaneta americana* in response to general odors were measured using a series of short odor pulses (20–400 ms). Odor pulses were delivered to olfactory sensilla in a moving airstream controlled by electromagnetic valves. The responses of sensory neurons were recorded using a tungsten electrode placed at the base of the sensillum. The temporal responses of sensory neurons followed the temporal changes in stimulus concentration, which were estimated by replacing the odorant with oil smoke and measuring the concentration of smoke passing through a light beam. Spike frequency varied with odorant concentration with surprisingly fine temporal resolution. Cockroach olfactory sensory neurons were able reliably to follow 25 ms pulses of the pure odorant 1-hexanol and 50 ms pulses of the complex odor blend coconut oil. Lower concentrations of odorants elicited responses with lower peak spike frequencies that still retained the temporal resolution of the stimulus pulses. Thus, responses of olfactory sensory neurons can reflect the fine structures of non-uniform distributions of general odorants in a turbulent odor plume as well as the average odorant concentration.

Key words: insect, olfaction, sensory coding, cockroach, *Periplaneta americana*.

Introduction

In the study of olfactory orientation in insects to general, non-pheromonal odors, temporal and spatial variation in odor plumes and the response characteristics of the peripheral olfactory system have been examined closely only recently. Odor plumes emanating from a point source are not uniformly dispersed. Owing to turbulent air flow, the odor is distributed in space as stretched filaments with increasing complexity as they move farther from the source (Kramer, 1986). The odor arrives at a location downwind of a point source in the form of brief pulses of odor separated by gaps of clean air (Murlis, 1986; Murlis and Jones, 1981). Similar turbulent odor plumes are also present in aquatic environments where pockets of odorant are separated by clean water (Atema, 1985; Moore and Atema, 1991). An animal moving through this chemical environment is faced with the difficult task of detecting and identifying odors that are presented for very brief periods at varying concentrations. This differs greatly from the typical experimental condition of presenting odors at constant concentrations for hundreds or thousands of milliseconds.

Studies of insect orientation to plumes of pheromone indicate that the pulses of odor present in a turbulent odor plume have an important effect on orientation behavior. Baker *et al.* (1985) showed that male oriental fruit moths *Grapholita molesta*, which do not fly upwind in a continuous uniform cloud of pheromone, did so when the pheromone was pulsed. Kramer (1986) exposed silkmoths, *Bombyx mori*, to 150 ms pulses of pheromone as well as to continuous pheromone. He found that, with pulses at repetition intervals of 0.3 s up to 0.7 s, the insects oriented more efficiently towards the source than they did with continuous stimulation. These experiments showed that fluctuations in the odor concentration were of behavioral significance.

The complexity of odor plumes (Murlis and Jones, 1981) presents something of a dilemma to sensory physiologists. Responses from single sensory cells, including adaptation (Kaissling *et al.* 1989; Rumbo and Kaissling, 1989; Zack-Strausfeld and Kaissling, 1986), had previously been measured using stimuli applied for the order of seconds. Considering the much shorter 10–100 ms pulses of odors observed by Murlis and Jones (1981), new experiments were needed to determine how individual receptors responded to short pulses. Rumbo and Kaissling (1989) measured the temporal response characteristics of three cell types of male *Antheraea polyphemus*, each responding to a different pheromone component, using series of short pheromone pulses. Two of

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three cell types sensitive to (*E*,*Z*)-6,11-hexadecadienal and (*E*,*Z*)-4,9-tetradecadienyl acetate were able to resolve at least 5 stimuli s^{-1}, whereas the third, responding to the major pheromone component (*E*,*Z*)-6,11-hexadecadienyl acetate, was slower, resolving only approximately 2 stimuli s^{-1}.

These studies and others illustrate how much attention has been paid to narrowly tuned receptor neurons specialized for the detection of pheromones (Masson and Mustaparta, 1990). Less attention has been paid to the detection of general odorants such as those associated with foliage (so-called 'green leaf odor'), flowers or other food items. Receptors sensitive to general odorants have been identified in insects such as the Colorado potato beetle *Leptinotarsa decemlineata* (De Jong and Visser, 1988; Ma and Visser, 1978), the American cockroach *Periplaneta americana* (Boeckh, 1976; Fujimura *et al.* 1991; Sass, 1976, 1978; Selzer, 1984) and the honeybee *Apis mellifera* (Akers and Getz, 1992, 1993; Getz and Akers, 1993, 1994, 1995; Vareschi, 1971). Generally, little is known about their response characteristics other than a broad evaluation of their response to suites of odorants given in long-duration pulses at single dosage levels.

The American cockroach *Periplaneta americana* provides an excellent model for the study of the temporal resolution of pulses of general odorants in insects. Its great advantage over the honeybee, for example, is that the predominant olfactory sensory structures, the basiconic sensilla, are innervated by only 1–5 neurons each (Schaller, 1978; Toh, 1977), and single-sensillum recordings from them show the activity of one or two receptor cells. Their counterparts on the honeybee, the placode sensilla, are innervated by 5–30 neurons each (Slifer and Sekhon, 1961), and single-sensillum recordings from them contain the activity of many neurons (Akers and Getz, 1992, 1993; Getz and Akers, 1993, 1994). Since only single-sensillum recordings are currently feasible, the problems with complex multi-unit recordings are greatly reduced with the cockroach.

If fluctuations in general odor concentration are important for orientation to food sources, it must be possible for insects to resolve pulsatile stimuli in order to distinguish them from constant stimuli. The purpose of the present study was to determine whether cockroach olfactory sensory neurons are able to resolve brief pulses of general 'green leaf' or other odors similar to those that animals may encounter in a turbulent atmosphere. Our predictions, based on the results from behavioral and physiological studies of pheromone reception in moths, show that cockroach olfactory neurons would be able to resolve pulses of odor with durations of 100 ms or less and that this limit of resolution would be dependent on stimulus concentration and quality. This study contributes to our goal of determining how insect nervous systems code odors in the real-world situation where complex stimuli of varying concentrations are encountered for varying, but inevitably brief, periods.

Materials and methods

Experimental animals and electrophysiological techniques

Our recording methods were similar to those used previously on honeybees, cockroaches and other insects (Akers and Getz, 1992, 1993; Fujimura *et al.* 1991; O'Connell, 1975; Sass, 1978; Selzer, 1984; Vareschi, 1971), and our stimulation methods closely followed those used by O'Connell and others (Akers and Getz, 1992, 1993; Akers and O'Connell, 1988; Grant and O'Connell, 1986; O'Connell, 1975).

Recordings were made from single sensilla of the female American cockroach *Periplaneta americana* (L.) obtained from Ward's (Rochester, NY, USA). Each cockroach was cold-anesthetized and secured ventral-side-up on a custom-built stage with tape across its abdomen and thorax. Small drops of melted wax were used to secure the top of its head to the stage. The left antenna was placed flat against a clear microscope slide and held in place with two narrow strips of clear plastic tape. The entire stage was placed beneath the objective of a Nikon Labophot microscope that rode on an *x–y* translation stage on the baseplate. The microscope was fitted with 10× and 40× long-working-distance objectives and 15× oculars. Epi-illumination was provided by a fiber-optic illuminator that allowed clear visualization of the sensilla. The cockroach has a variety of sensillum types (Fujimura *et al.* 1991; Schaller, 1978). The sensilla from which we recorded were basiconic sensilla, which are among the smallest cockroach antennal sensilla with diameters of 1–2 μm and lengths of 8–10 μm. In the female American cockroach, each of the basiconic sensilla is known to be innervated by 1–4 neurons (Fujimura *et al.* 1991; Schaller, 1978). These sensilla typically do not contain any mechanosensory neurons and, to confirm this, recordings were made from each sensillum when presented with several stimulus pulses with no odorant.

The electrodes were fashioned from 125 μm tungsten wire, electrolytically sharpened to less than 1 μm at the tip. The reference electrode was placed in the tip of the antenna. The recording electrode was placed near the base of a sensillum using a Narishige MO-202 joystick manipulator. Sensilla were sampled widely from all parts of many antennal segments. Signal size ranged from approximately 40 mV to 200 mV peak-to-peak. Signals were amplified differentially through a WPI DAM-50 amplifier, with a bandpass of 300–1000 Hz. The signal was then fed into an oscilloscope and an analog-to-digital (A/D) converter, stored on a microcomputer and analyzed using SAPID Tools (Smith *et al.* 1990). Recordings began 0.5 s before the stimulus was delivered and were 3.0 s in duration. The analog signals were digitized at a sample rate of 10 kHz. Owing to problems inherent to the spike-sorting process, spikes were not sorted into different classes, but were treated as a single spike class.

Stimulation techniques

Two streams of air were aligned facing each other on either side of the antenna, with the air emerging from plastic tubing (2 mm inside diameter) approximately 5 mm from the sensilla. A clean air stream, or purge, flowed continuously over the target sensillum at approximately 210 mm min^{-1}, except during stimulation. The stimulus airstream was at approximately 60 ml min^{-1}, and stimuli were delivered by passing this

airstream through a cartridge containing odorants dissolved in 1 μl of mineral oil placed on a 7 mm×35 mm slip of Whatman no. 1 filter paper. Both airstreams came from compressed house air filtered through DriRite and a 0.5 nm molecular sieve (gas purifier model L68GP, Cole-Parmer).

Control of stimulation of the antenna was integrated with the A/D data acquisition on the microcomputer. Both airstreams were controlled through the computer by miniature Teflon solenoid valves (Lee Co., Westbrook, CT, USA). In all stimulations, the stimulus line was first turned on for 500 ms with the purge still on (Grant and O'Connell, 1986). This flushed the stimulus cartridge with approximately two volumes of clean air. After this 500 ms period, the purge stream was immediately turned off, allowing the odorant to reach and stimulate the sensillum. To produce pulsatile stimuli, the purge airstream was turned off and on at appropriate intervals while the stimulus airstream remained on continuously. This produced a train of odor pulses separated by clean air. At the end of the stimulation, both the purge and the stimulus streams were turned off. Stimulus trains were generally presented at 1–2 min intervals.

The responses from two groups of 20 cockroaches were recorded. One group was presented with longer-duration pulses (⩾100 ms) and one group was presented with shorter-duration pulses (⩽100 ms). In the longer-duration group, each odorant was presented to each sensillum at two concentrations in 10 different stimulus trains. The entire set of stimulus trains included an initial 2000 ms pulse, followed by a set of pulsatile stimulus trains. Each pulsatile train was described by the duration of each odor pulse and the duration of the interval between pulses, and pulses were presented to the long-duration group in the following order: 100 ms/100 ms, 200 ms/200 ms, 400 ms/400 ms. These pulsatile trains were followed by a second 2000 ms stimulus to assess the health of the responding neurons and to control for long-term adaptation. In the shorter-duration group, each odorant was presented to each sensillum at two concentrations in six different stimulus trains. This group received an initial 2000 ms pulse followed by five pulsatile stimulus trains (20 ms/20 ms, 25 ms/25 ms, 50 ms/50 ms, 75 ms/75 ms) and a second 2000 ms pulse.

Two sets of odorants were used, the pure odorant 1-hexanol and the complex blend of odorants coconut oil, each presented at concentrations of 10 and 100 $\mu g\,\mu l^{-1}$. The more complex coconut odor was included in this study because previous results have indicated that complex blends of odorants elicit intracellular or intercellular inhibitory responses in insect chemoreceptors (Getz and Akers, 1995; White *et al.* 1990). These inhibitory interactions may have a profound effect on the temporal responses to complex odorants. The reported concentrations represent the concentration of the odorant in 1 μl of mineral oil placed on the filter paper in the odor cartridge. The actual concentration of odorant in the air was much lower and dependent on the rate of evaporation of the odorant into the moving airstream during stimulus delivery. Each of 20 sensilla received the entire series of long-duration stimuli, and another set of 20 sensilla received the entire series of short-duration stimuli. Each sensillum was also presented with several control stimuli that contained mineral oil only in addition to blank stimuli of clean air only. None of the basiconic sensilla included in the results of this paper responded to these blank or control stimuli.

For long pulses (⩾100 ms), the responses were divided into 50 ms time bins and the total number of spikes that occurred during each bin was counted. The 50 ms bin width was chosen to allow sampling at twice the frequency of the shortest stimulus pulse, ensuring that the resolution of the recording and the analysis would exceed that required to detect pulse resolution by the sensory neurons. Owing to problems inherent in the spike-sorting process, spikes were not sorted into different classes, so the spike frequencies calculated were the summed responses of all the responding neurons within each sensillum. In most cases, only one neuron responded in each sensillum, but in rare cases as many as three responded. For shorter pulses (⩽75 ms), the responses were divided into 10 ms time bins and the number of spikes in each bin was counted. Again, this bin width provided a sampling frequency twice that of the shortest stimulus pulse, ensuring adequate resolving power of the analysis to detect pulse resolution by the sensory neurons. The mean of the responses (± S.E.M.) from 20 separate sensilla that responded to both 1-hexanol and coconut oil was calculated for each type of stimulus train.

Stimulus characterization

To characterize accurately the time course and concentration of each stimulus pulse, we used smoke as a substitute for the odorants and measured the concentration of smoke with a photodetector as it passed through a beam of light. The odor cartridge was replaced with an aluminum tube of the same diameter heated to approximately 200 °C. A drop of mineral oil placed in the tube slowly evaporated, producing dense white smoke. The smoke was blown from the tube using the same system of valves and pressurized air that was used to deliver odor stimuli. An infrared-light-emitting diode was placed just below the position at which the cockroach sensillum would otherwise be, and the light was directed up through a 1 mm×1 mm clear window in an opaque, black microscope slide. The smoke was directed at the clear window in a manner that approximated the direction of the odorant towards the cockroach sensillum. An infrared phototransistor was placed 1 cm above the slide and detected the light emitted from the light-emitting diode through the clear window, converting the light intensity into a voltage. Smoke blowing across the clear window reflected the infrared light and changed the voltage produced by the phototransistor. The voltage produced by the phototransistor, which represented smoke density, was amplified and recorded as described above for extracellular recordings of sensory neuron activity and reflected the change in smoke density through time. Smoke density was measured with a sampling rate of 10 kHz. Each of

the stimulus trains was reproduced five times, and the mean smoke density (± S.E.M.) was calculated for each stimulus paradigm. The graphs of smoke density were assumed to be accurate estimates of the time courses of the onsets and offsets of each odor pulse and the variation in odor concentration during each pulse.

Results

Stimulus characterization

Interpretation of the temporal response characteristics of olfactory receptor neurons depends on an accurate knowledge of the changes in odorant concentration at the sensillum. These are not equivalent to the times at which the valves controlling the flow of clean air through the purge line open and close. To determine the arrival times of odorant and clean air at the sensillum, we replaced the odorants with smoke and measured its concentration using a phototransistor at the point where the sensillum would be located. The normalized mean smoke density (± S.E.M.) as a function of time indicated that the onsets and offsets of the stimulus, as determined by its arrival at the sensillum, were extremely abrupt, nearly approximating square waves in the longer pulses (Fig. 1). There was also a substantial delay of 50–100 ms between the time the valve opened and the arrival of the stimulus, which can be accounted for by the distance that the air traveled through the tubing between the valve and the sensillum. In the stimulus trains with the shortest pulses (20–25 ms), smoke density did not return to baseline between each pulse. There was an increasing background smoke concentration upon which the pulses rode. This gradual increase, which was most evident in the 20 ms pulse trains, may have been responsible for the increase in spike frequency and the change in pulse resolution seen in the physiological responses to these stimulus paradigms. There was also a sharp increase in smoke concentration at the end of the stimulus train when the stimulus and purge air flows were stopped simultaneously (Fig. 1). In no case was there a corresponding physiological response to a sharp increase in odorant concentration, so it appears that this large terminal smoke pulse was an artifact of the smoke measurement or delivery techniques.

Temporal resolution of odor pulses

Records from basiconic sensilla typically showed a very low level of spontaneous activity (Fig. 2A). Stimulation with clean air or mineral oil only rarely elicited any recognizable response. Only a small proportion of the sensilla sampled responded to both 1-hexanol and coconut oil. Of those, approximately 10 % were incapable of resolving the onset or offset of the longest stimulus pulses delivered (2000 ms). Those neurons that responded to the stimuli but did not resolve onsets or offsets typically began firing 500–1000 ms after the stimulus onsets and continued firing for 500–2000 ms after stimulus offset. Responses from these types of cells were not

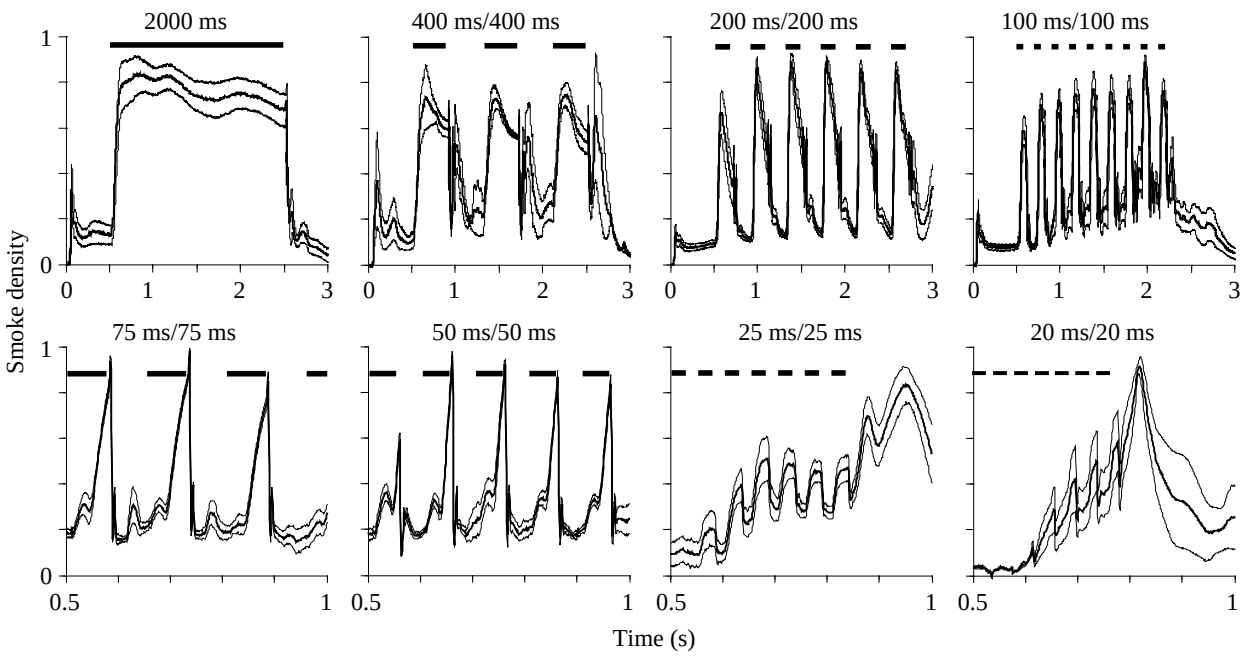

Fig. 1. Normalized smoke density for each of the stimulus trains with pulse durations ⩾100 ms (upper row) and ⩽75 ms (lower row) used to deliver odors to olfactory neurons. The source of mineral oil smoke replaced the odor cartridge and the density of smoke was measured using a photodetector. The smoke was analogous to the odorant molecules and the time course of variation in smoke density was assumed accurately to reflect the time course of variation in the concentration of odorants. Each figure shows the mean of five smoke deliveries (thick line) and the mean ± S.E.M. (thin lines). Note that the time scale differs for the stimulus trains with the four shortest pulse durations (lower row). The duration of the smoke pulses and the duration of the intervals between them are shown by the first and second numbers above each graph, respectively. The thick horizontal bars in each graph represent the times that the purge airflow was off, allowing the smoke to reach the sensillum.

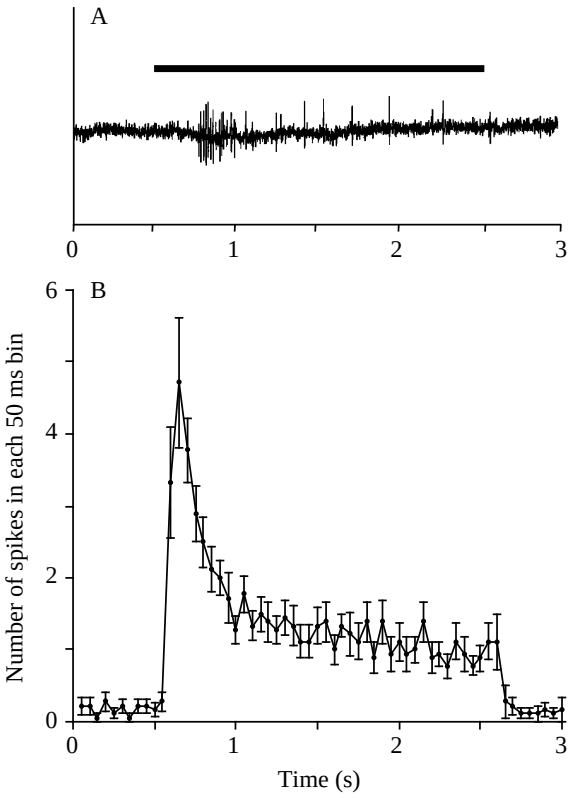

Fig. 2. (A) Typical spike train recorded in response to a long-duration (2000 ms) pulse of 1-hexanol. The time course of the stimulus is represented by the black bar. This train contained the activity of at least two neurons. (B) The mean number of spikes (± S.E.M.) in each 50 ms time bin for 20 spike trains in response to the stimulus that evoked the response shown in A. This figure, and many of the following figures, shows the 500 ms preceding the stimulus onset and the following 2500 ms. Note the rapid changes in spike frequency that correspond to the stimulus onset and offset.

analyzed. All the data presented here are means (± S.E.M.) of 20 basiconic sensilla that responded to both 1-hexanol and coconut oil and had physiological responses to the 2000 ms pulse of odor that began less than 500 ms after stimulus onset (Fig. 2B).

The changes in spike frequency in response to the longer-duration odorant pulses (⩾100 ms) followed the onsets and offsets of odor stimuli very well (Figs 3–6). During the longest stimulus pulses (400 and 2000 ms), the spike frequency showed both phasic and tonic characteristics, with the initial phasic period continuing for 300 ms or longer. These data suggest that there may have been some short-term adaptation during the longer odor pulses. In addition, the difference in peak spike frequency between the responses to the first 2000 ms pulse, given before all the pulsatile stimulus trains, and the second 2000 ms pulse, given 60–90 min later after all the pulsatile stimuli, suggests that there was also some form of long-term adaptation occurring.

Resolution of odor pulses down to 100 ms in duration did not appear to be dependent on odorant concentration. Peak spike frequencies of responses elicited by pulses of 100 μg μl^{-1}

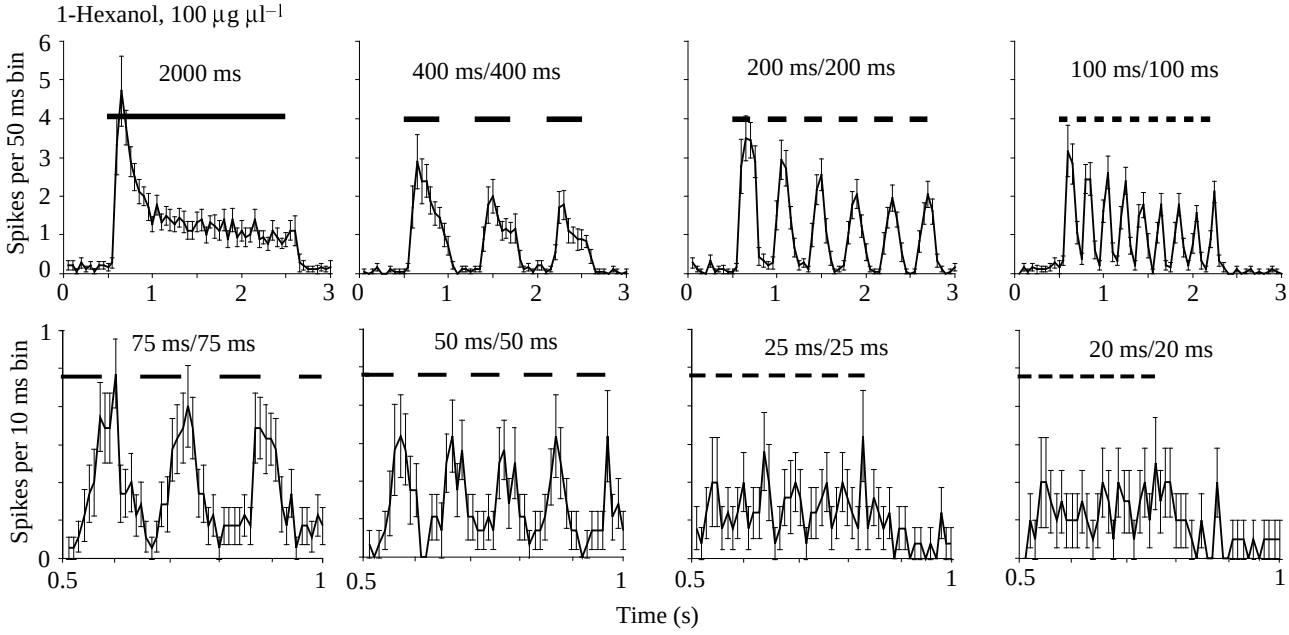

Fig. 3. Mean number of spikes in each time bin (± S.E.M.) evoked by stimulation with 100 μg μl^{-1} 1-hexanol. This upper row shows the responses to the stimulus trains with long-duration (⩾100 ms) pulses. The bin width used for analysis of the responses to long-duration pulses was 50 ms. The lower row shows the mean number of spikes in each 10 ms bin (± S.E.M.) evoked by stimulus trains with short-duration (⩽75 ms) odor pulses. In this and subsequent figures, the duration of the odor pulses and the duration of the intervals between them are shown by the first and second numbers above each graph, respectively. The thick horizontal bars in each graph represent the times that the purge airflow was off, allowing the stimulus to reach the sensillum.

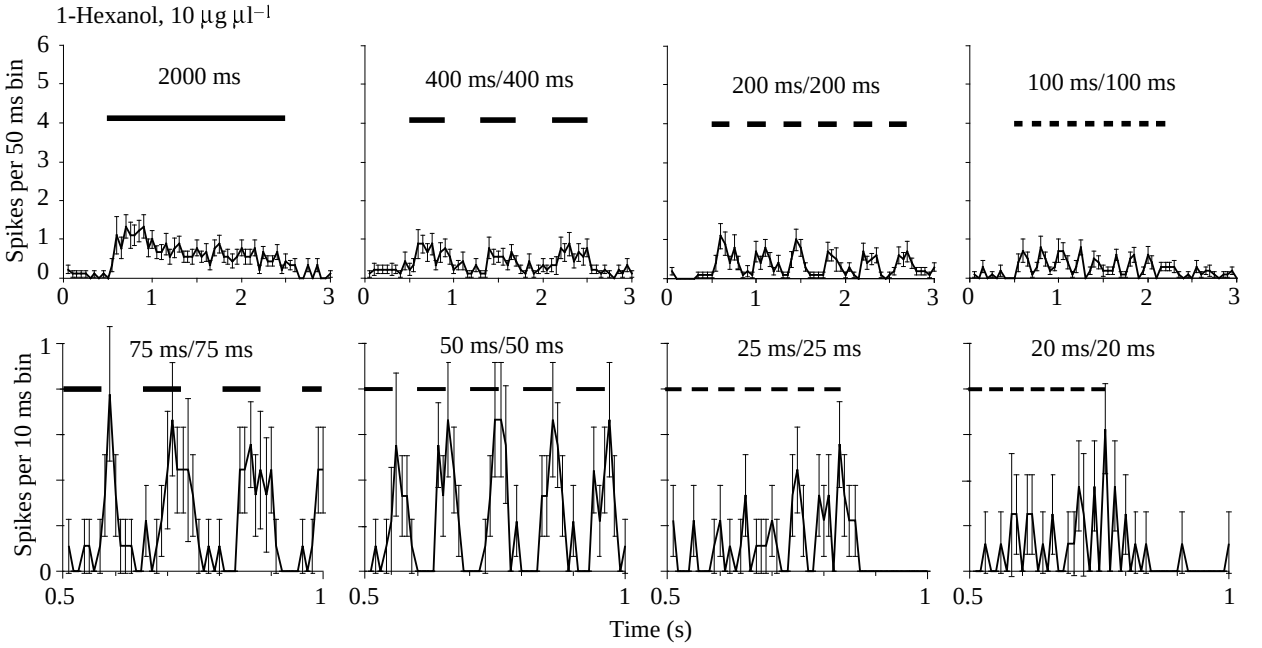

Fig. 4. Mean number of spikes in each 50 ms bin (upper row) or 10 ms bin (lower row) evoked by stimulation with 10 μg μl^{-1} 1-hexanol. Other details are as in Fig. 3.

1-hexanol (Fig. 3) were substantially higher than those elicited by pulses of 10 μg μl^{-1} 1-hexanol (Fig. 4), but there were still distinct increases in spike frequency at the lower odorant concentration corresponding to the onset of stimulation and decreases in spike frequency corresponding to the offset of stimulation.

The temporal resolution of short-duration (≤75 ms) pulses of odor appears to be dependent on odor quality and concentration (Figs 3, 4). The responses to 75 ms and 50 ms pulses of 1-hexanol were distinctly correlated with the onsets and offsets of pulses of both odorant concentrations (10 μg μl^{-1} and 100 μg μl^{-1}). When pulse duration was reduced to 25 ms, responses to 100 μg μl^{-1} 1-hexanol continued to show an increase in firing rate corresponding to the presence of the odorant, but inconsistently resolved the periods of clean air between pulses. This suggests that the dynamics of stimulus transduction were reaching their limit of temporal resolution and that neurons in this environment began to perceive this stimulus as one constant pulse. The same stimulus paradigm at 10 μg μl^{-1} 1-hexanol evoked several other notable phenomena (Fig. 4). The low concentration of odorant and the brief pulses were barely able to evoke a response that was above threshold. As the stimulus train progressed, however, the peak spike frequency increased, so that the last few pulses were well resolved. This increase in resolution suggests that there may have been some temporal summation of these low-amplitude responses or that the short-duration pulses were only able to produce a stimulus concentration above threshold after the pulses had been added to an increasing background concentration. This result is in accordance with results obtained by Getz and Akers (1997) suggesting that, in American cockroaches, the response during the interval from 150 to 250 ms after stimulus onset provided more robust coding at lower concentrations, while the response during the interval from 50 to 150 ms after stimulus onset provided more robust coding at higher concentrations. A similar pattern was seen in the responses to 20 ms pulses of both concentrations of 1-hexanol, but suggested a different interpretation. Although spike frequency increased as the stimulus train progressed, neither response resolved these extremely brief pulses. This gradual increase in spike frequency without resolution of individual pulses suggests that the increase in spike frequency was due only to the increase in background odorant concentration but did not reach the threshold for pulse discrimination.

The differences in peak spike frequency to high and low concentrations of the complex blend of odorants in coconut oil are more difficult to interpret (Figs 5, 6). Previous studies of the responses of olfactory sensory neurons to blends of odorants indicate that mixtures could inhibit responses, resulting in lower spike frequencies than are evoked by the pure odorants in the mixture (Akers and Getz, 1993; Getz and Akers, 1995). Our results are consistent with that conclusion. The peak spike frequencies evoked by stimulation with 10 μg μl^{-1} coconut oil (Fig. 6) equaled or exceeded those evoked by 100 μg μl^{-1} coconut oil (Fig. 5). These data suggest that the lower concentration of odorants may have elicited less of the inhibitory interaction that is believed to produce the lower spike frequencies in responses to mixtures. In addition, the phasic portion of the response to the second 2000 ms odor pulse had a higher peak frequency than that of the first 2000 ms odor pulse. This suggests that long-term adaptation of an inhibitory process may have released the neurons from inhibition after 60–90 min of pulsatile stimulation.

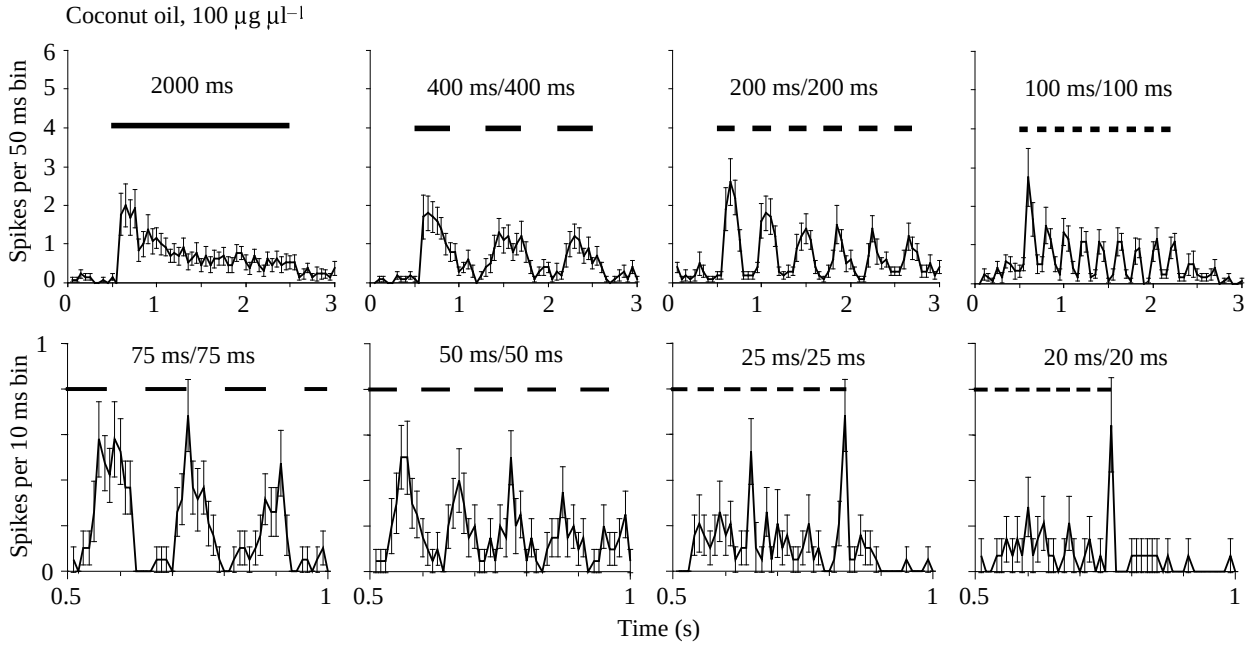

Fig. 5. Mean number of spikes in each 50 ms bin (upper row) or 10 ms bin (lower row) evoked by stimulation with 100 μg μl^{-1} coconut oil. Other details are as in Fig. 3.

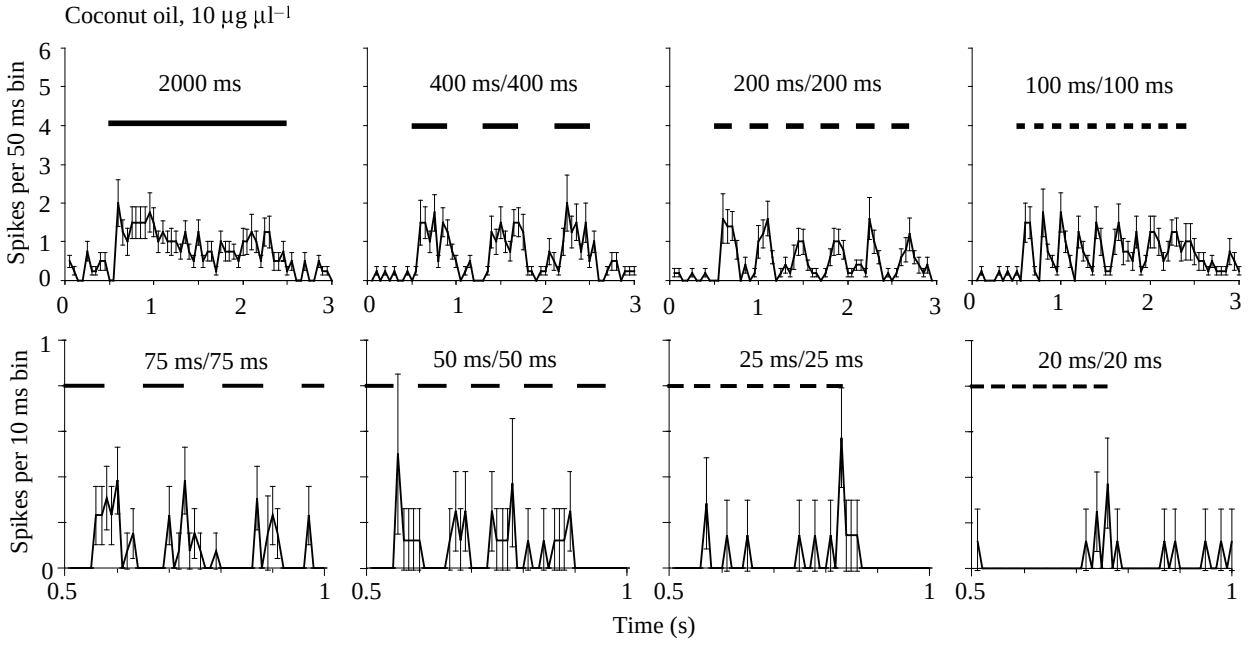

Fig. 6. Mean number of spikes in each 50 ms bin (upper row) or 10 ms bin (lower row) evoked by stimulation with 10 μg μl^{-1} coconut oil. Other details are as in Fig. 3.

The responses to short pulses (≤75 ms) of coconut oil (Figs 5, 6) were similar to those evoked by 1-hexanol (Figs 3, 4) with one major difference: the responses to coconut oil were unable to resolve 20 or 25 ms pulses at either concentration. At the lower concentration of coconut oil, the responses only poorly resolved 50 ms pulses. With pulses shorter than 50 ms, the spike frequencies were only occasionally distinguishable from the background firing rate. These data indicate that the dynamics of the neuronal response to the complex blend of odorants in coconut oil were slower than the dynamics of responses to 1-hexanol, which may also have been the result of inhibition due to the presence of multiple odorants.

By superimposing the smoke density results and physiological responses, it is evident that most of the delay in the physiological response would be due to the delay in the arrival of the odorant at the sensillum, if the smoke profiles

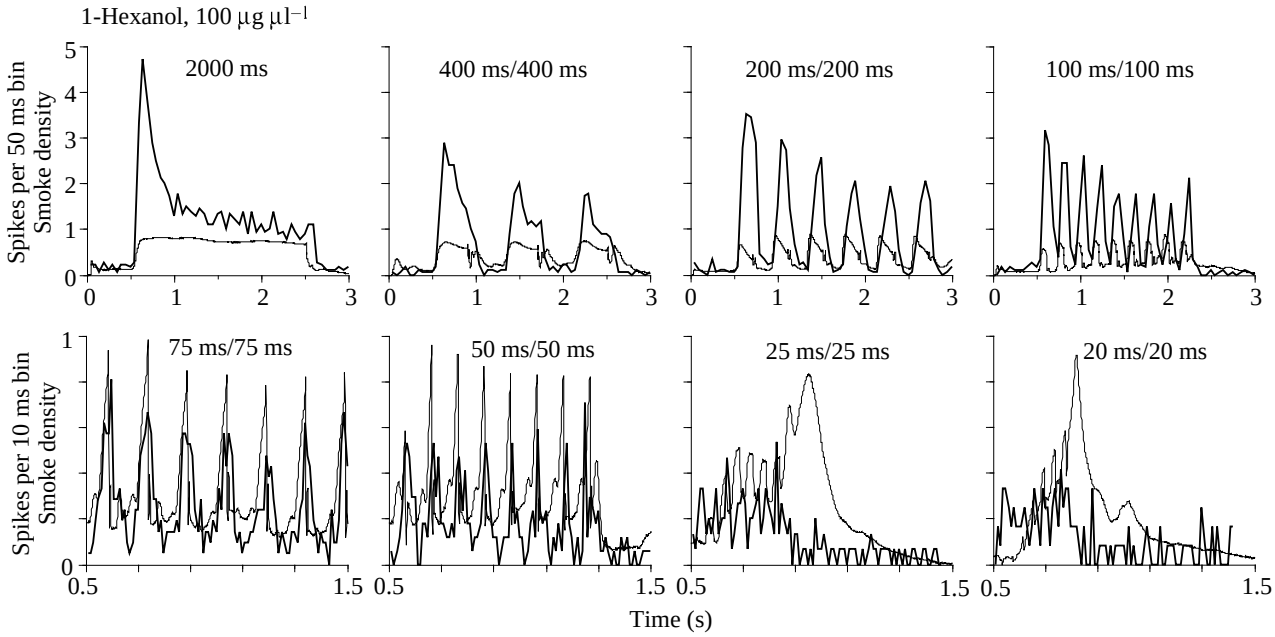

Fig. 7. Mean number of spikes in each 50 ms bin (thick lines; upper row) or each 10 ms bin (thick lines; lower row) evoked by stimulation with 100 μg μl^{-1} 1-hexanol. The thin lines in each graph represent the mean smoke density for the corresponding stimulus train and are analogous to the odorant concentration. These graphs more accurately show the arrival of the odorant at the sensillum and the changes in odorant concentration. The corresponding delays in onset and offset of the physiological response and the changes in spike frequency are indicative of the temporal response characteristics of the sensory neurons. There is good resolution of odor pulses down to a stimulus/pause time of 25 ms/25 ms. Other details are as in Fig. 3.

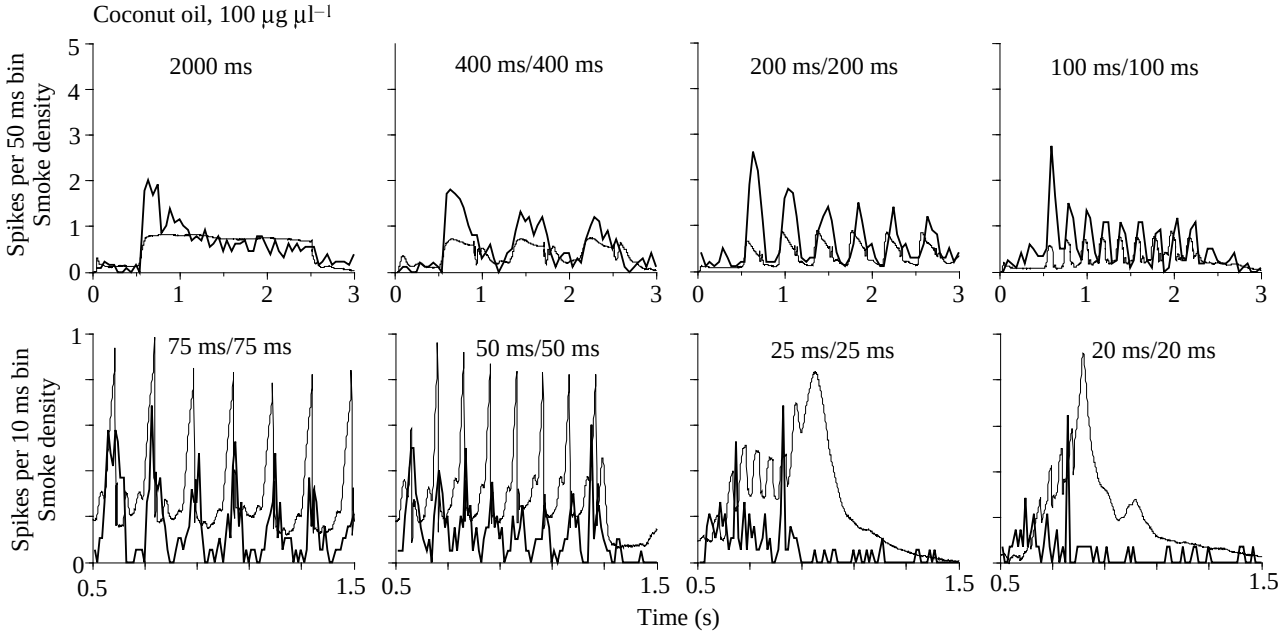

Fig. 8. Mean number of spikes in each 50 ms bin (thick lines; upper row) or each 10 ms bin (thick lines; lower row) evoked by stimulation with 100 μg μl^{-1} coconut oil. The thin lines in each graph represent the mean smoke density for the corresponding stimulus train and are analogous to the odorant concentration. There is good resolution of odor pulses down to a stimulus/pause time of 50 ms/50 ms. The onset of the first physiological response coincides with the increase in odorant concentration, but subsequent physiological responses tend to begin after an increasingly long delay.

provide accurate representations of the odor stimulus profiles. In the responses to stimulus trains with longer pulses (⩾100 ms), the onsets of the odor pulse and the onsets of the physiological response were indistinguishable, which indicates that the delay was less than the limit of resolution imposed by the 50 ms bins (Figs 7, 8). In those stimulus paradigms using

shorter odor pulses where responses always resolved the stimulus pulses (50 and 75 ms), the delays between the arrival of the first few pulses of odorant and the beginning of the responses were less than 40 ms. Subsequent pulses evoked responses with variable, but generally increasing, delays. At the shortest pulse duration (20 ms), the sensory neurons did not respond fast enough to stimulus onsets and offsets to keep up with the pulse train.

Discussion

Temporal response characteristics of olfactory receptors

The results of this study show that olfactory sensory neurons in the antennae of the American cockroach *Periplaneta americana* that are sensitive to general odors are able to resolve pulsatile stimuli of 1-hexanol up to rates of 40 pulses s^{-1} (25 ms pulses) and are able to resolve pulsatile stimuli of coconut oil up to rates of 20 pulses s^{-1} (50 ms pulses). These maximum rates appeared to be relatively independent of odor concentration, provided that the concentration and pulse duration produced a stimulus that was above threshold. These resolution frequencies are substantially faster than those previously reported for pheromone-sensitive neurons in moths. Rumbo and Kaissling (1989) found that pheromone receptor cells in male *Antheraea polyphemus* were able to resolve pulsatile stimuli at rates up to 10 stimuli s^{-1} (100 ms pulses), and Christensen and Hildebrand (1988) found that projection neurons in the central nervous system of *Manduca sexta* were also able to resolve 10 stimuli s^{-1}. Our results suggest that insects orienting towards a source of plant-type odors are able to evaluate fluctuations in concentration on a finer time scale than they are able to evaluate fluctuations in pheromone concentration. This difference may be because pheromone receptors are adapted to respond to much lower concentrations of odors than are general odor receptors (Fujimura *et al.* 1991; Sass, 1983).

Behavioral studies of orientation of moths to pheromones have shown repeatedly that pulsed or turbulent odor plumes are more effective at evoking upwind orientation behavior than are more constant stimuli (Baker *et al.* 1985; Kramer, 1986; Willis *et al.* 1994). Similarly, female moths orienting to host plant odors require a turbulent odor plume to stimulate upwind zigzagging flight (Willis and Arbas, 1991). This suggests that the detection and localization of very brief pulses of odor, provided that they are long enough for the animal to extract enough information from the signal to identify the odorant, may play a significant role in the foraging behavior of cockroaches.

The problem of identifying odorants occurring in brief, pulsatile stimuli was indirectly addressed in a recent study of coding efficiency in cockroach olfactory neurons. Getz and Akers (1997) compared the response patterns of the small ensemble of neurons within a sensillum to a variety of hexyl and heptyl odorants. By using cluster analysis, they generated response templates from these data that represented the typical ensemble response that discriminated each odorant. To determine which portion of each response provided the best information, the templates were then compared with ensemble responses during the first 100 ms of the response, which began 50 ms after the stimulus onset, the second 100 ms of the response and the response during the entire 500 ms duration of the stimulus. In general, the proportion of correct identifications of stimuli was greatest when the information from the first 100 ms of the response was used. Their results suggest that cockroaches were able to discriminate reliably between closely related odorants within 100 ms of exposure. The results of the current study indicate that peak spike frequencies comparable with those evoked by 100 ms pulses could be obtained with stimuli as brief as 50 ms, suggesting that general odor discrimination may be possible within 50 ms of exposure.

Response characteristics of generalist olfactory receptors

Every sensillum included in this study was capable of resolving 25–50 ms pulses of both 1-hexanol and coconut oil and most responded with the activity of two or more neurons. Because each basiconic sensillum contains only 1–5 sensory neurons, the generalist sensory neurons represented in these recordings were probably broadly tuned and responded to both odorants. The broad tuning characteristics of each olfactory neuron are probably caused by olfactory neurons expressing several, and perhaps many, different populations of protein receptor types in their membranes (Lancet and Ben-Arie, 1993; Lancet *et al.* 1993; Ngai *et al.* 1993). However, activation of multiple receptors does not result in an additive response in the neuron. In the results of our experiments, the peak spike frequencies evoked by stimulation with 100 $\mu g\,\mu l^{-1}$ coconut oil were lower than those evoked by 10 $\mu g\,\mu l^{-1}$ coconut oil. Studies of cockroach olfactory neurons with mixtures of odorants indicated that responses to blends resulted in lower spike frequencies than were evoked by pure odorants (Akers and Getz, 1993). Stimulation with complex odor blends may activate a variety of inhibitory processes that can result in a decreased response amplitude. Insect chemosensory neurons, some of which may have inhibitory responses to stimulation, have been shown to interact in the periphery (Getz and Akers, 1994; White *et al.* 1990). Inhibition may also occur within a single sensory neuron. Several different second messenger systems have been identified in chemosensory neurons (Michel *et al.* 1991), some of which lead to the activation of depolarizing Na^+ channels and others to the activation of hyperpolarizing K^+ channels. This interplay of activated Na^+ and K^+ channels may produce decreases in spike frequency with increasing stimulus concentration and nonlinear responses to mixtures (Daniel and Derby, 1987; Daniel *et al.* 1994; Getz and Akers, 1995). The paradoxical result of the current study, in which a low concentration of coconut oil evoked higher spike frequency responses than did a higher concentration, suggests that the lower concentration may have elicited less of the inhibitory interaction that produces lower spike frequencies in response to mixtures.

Temporal resolution in the central nervous system

The results described in this paper provide evidence of the limit of temporal resolution of olfactory stimuli in the peripheral nervous system. Odor identification and discrimination, however, are believed to be carried out in the complex olfactory neuropil of the central nervous system (Hildebrand, 1996). The information pathway in typical insect olfactory systems begins with tens of thousands of sensory neurons in the sensilla on the antennae that converge on a hundred, or fewer, ball-like neuropil structures known as glomeruli located in the olfactory lobe of the deutocerebrum. Here they arborize and form neural networks with interneurons possessing extensive intra- and inter-glomerular projections (Boeckh *et al.* 1984; Christensen and Hildebrand, 1987; Matsumoto and Hildebrand, 1981; Olberg, 1983; Suzuki and Tateda, 1974). Relay neurons then project from the glomeruli along several tracts that convey olfactory information to higher multimodal memory and learning centers of the brain (Buonviso and Chaput, 1990; Getz, 1994; Kauer and Moulton, 1974; Osorio *et al.* 1994; Wellis and Scott, 1990). The temporal resolution of olfactory stimuli possible in these complex higher centers, involving large networks of interneurons, may be on a much slower time scale than the temporal resolution possible in the primary sensory neurons. The temporal response characteristics of the sensory neurons only set the lower limit of the duration of odor pulses that the organism would be able to identify.

Our results indicate that cockroach olfactory neurons that are responsive to both 1-hexanol and coconut oil can resolve pulses as short as 25 ms. Sensory neurons that are responsive to only one odorant, however, may be able to resolve much shorter pulses. In addition, the population of sensilla that we sampled was small compared with the thousands of sensilla on each antenna. By increasing the size of the sampled population, we might include neurons that have much faster temporal response characteristics, and the cockroach's ability to resolve extremely short pulses of odor may become apparent. The inputs from many olfactory neurons with different temporal response characteristics that respond to a single odorant may also provide adequate temporal information to the antennal lobe to allow resolution of shorter pulses. The results of this study show that cockroach olfactory sensory neurons can resolve pulsatile stimuli that occur at frequencies of at least 40 Hz, but cockroaches may have the ability to resolve pulses at much higher frequencies.

The results of these experiments show that sensory neurons are capable of resolving brief, pulsatile odor stimuli, but they do not address the question of whether brief bursts of sensory neuron activity contain adequate information for the central nervous system to discriminate closely related odors. Experiments to determine the information content of pulsatile responses would involve decomposing the multi-unit responses from several sensilla into the responses of each of the individual neurons to create ensemble response patterns that faithfully represent stimulus quality. Then the ensemble responses to long-duration stimuli would be compared with the ensemble responses to brief pulsatile stimuli. This may indicate whether enough information is preserved in responses evoked with pulsatile stimuli to allow discrimination of different odors.

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